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Diagnostic methods for *H. pylori* infection: Choices, opportunities and pitfalls

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Helicobacter pylori resides in its specific niche, the stomach, colonizes the gastric epithelium, and causes chronic active gastritis with a resulting broad spectrum of possible complications.[1] There is no other chronic infection in the gastrointestinal tract nor elsewhere for which a comparable set of diagnostic methods is available as in the case of *H. pylori* infection.

The bacterium is detectable virtually in all biological samples, including gastric mucosa samples, its site of residence, but also in saliva, breath, blood, feces and even in urine. Means of detection include direct histomorphological visualization, culture, measurement of enzyme activity (i.e. urease) and metabolic products of urea degradation, detection of antigens, polymerase chain reaction (PCR), and antibodies as the result of the systemic immune response.

In clinical practice the most meaningful tests are those that indicate current infection with an immediate and direct impact on treatment. Diagnostic procedures are based either on invasive methods, such as gastric biopsies for rapid urease detection (rapid urease test (RUT)), histology and culture or on noninvasive methods, the 13C-urea breath test (13C-UBT) or fecal antigen determination (fecal antigen test (FAT)).[2,3]

Each of these tests if properly performed guarantees high diagnostic accuracy, and a positive result in each single one of these tests should lead to the induction of eradication therapy. Furthermore each individual gastric biopsy-based test offers a specific clinical advantage. The RUT provides the quickest result with the option to start treatment with no delay; the histological examination provides a comprehensive assessment of the gastric mucosa with an impact on short- and long-term management. Culture has the highest specificity but some limitations regarding sensitivity as it requires special care in handling of the mucosal specimen. Culture is the method of choice for antibiotic susceptibility testing and is of critical importance for the selection of adequate eradication therapies in times of increasing antibiotic resistance. Real-time PCR is an emerging option for bacterial resistance testing directly in gastric biopsies without requiring culture.[4,5]

The noninvasive 13C-UBT and FAT are of comparable diagnostic accuracy with biopsy-based tests and are the methods of choice in the test and treat setting and for controlling the effect of eradication treatment.[6]

Serology with determination of immunoglobulin G (IgG) *H. pylori* antibodies if properly validated shares the high diagnostic accuracy with biopsy-based and noninvasive tests, but does not allow the discrimination of whether the *H. pylori* infection is current or past and no longer persistent. A useful additional tool in serological tests is the determination of gastric functional parameters (i.e. pepsinogens, gastrin) that allow a good estimate of the condition of the gastric mucosa and in particular regarding the presence of severe atrophic changes.[7]

All tests used for *H. pylori* detection need to be considered individually for their advantages and disadvantages in diverse clinical conditions and settings and their use should be made accordingly. Selection and interpretation of test results need to respect specific conditions such as peptic ulcer bleeding, atrophic gastritis with/without intestinal metaplasia and the impact of diverse medication such as proton pump inhibitors (PPIs), antibiotics and bismuth salts.

In this issue of the *United European Gastroenterology Journal* (*UEG*), two original articles report relevant aspects concerning *H. pylori* testing.

The article by Parihar et al.[8] addresses the question whether in clinical practice a single biopsy from the antrum for RUT is sufficient or whether two biopsies from two sites (including the corpus) for RUT provide a higher sensitivity. Their finding is that taking two biopsies, one from the antrum, one from the corpus and combining them in one gel-chamber

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increases the detection rate of *H. pylori* infection with a number needed to be tested for an additional diagnosis of four.

The sensitivity of a single positive RUT from the antral biopsy moves from 57% up to 84% if two biopsies are taken. The contribution of a single biopsy if taken from the corpus is not reported, but would be of interest. The higher sensitivity of two biopsies is expected since a focal distribution of bacteria in the gastric mucosa is recognized and previous studies reported an increased accuracy of RUT if based on more than one biopsy.[9] The sensitivity of 84% is low. In an earlier study, a sensitivity of 90% was obtained from a single biopsy in the antrum for RUT in treatment-naive patients and only a 3% gain was found with an additional biopsy sample placed in a separate gel-chamber.[10] The significant increase by combining antral and corpus biopsies in this study[8] may in some part be due to PPIs. The fact that patients were requested to stop the intake of PPIs 10 days prior to undergoing the test procedure is not a guarantee and hidden PPI intake is an issue. Acid suppression with PPI shifts *H. pylori* toward the corpus and fundus mucosa with an increasing but still low colonization gradient, whereas the antrum gets cleared of *H. pylori* in 40% up to 80% of patients on PPIs.[11]

If PPI intake is reported or suspected, two biopsies for RUT would accordingly be advised from the corpus and fundus mucosa rather than from the antrum. Five days high-dose PPIs (i.e. omeprazole) have a significant effect on urease activity (RUT and 13C-UBT), therefore we need to carefully consider PPI use and its duration. Less than five days high-dose PPI (i.e. omeprazole 80 mg) have no significant effect on urease activity as measured by the 13C-UBT[12] and the use of pantoprazole had no effect over a period of 14 days in one study.[13] For all other PPIs, a seven-day withdrawal of PPI was necessary to obtain reliable test results. Current guidelines recommend a ''safety interval'' of 14 days. The practical consequence at present is that if a patient presents to gastroduodenoscopy on PPI, biopsies for RUT from the fundus-corpus mucosa combined with the histological examination are recommended (with culture as further option). Other causes for the low sensitivity in the study reported in this issue of the UEG journal may be the short reacting time of 30 minutes, which should be extended to several, at least eight, hours (according to our experience) as the low density of bacterial colonization requires more time for enzyme reaction. Attention should also be paid to the sequence of biopsy sampling. The formalin contaminated forceps, after sampling for histology should be avoided.

For eradication control, the ''combined antral/corpus biopsy'' RUT as suggested in the paper is not the preferred method. Severe pathologies require endoscopic follow-up with extensive histological examinations that include the detection of residual bacteria. In all other clinical conditions the domain for controlling the eradication effect is for noninvasive testing with the methods of choice, 13C-UBT and FAT.

An opportunity for the RUT is the further use of tissue taken from the test chamber for further molecular analysis.[9]

The study by Ramirez Lazaro et al.[14] reports on a significant rate of ''false-positive'' 13C-UBTs with the claim that a number of these are interpreted erroneously as false as they may rather indicate an occult *H. pylori* infection. According to these authors, elevating the cutoff delta over basal (DOB) for discrimination between positive and negative tests would reduce the test performance and thus they advise keeping the lower cutoff. We share the concept that a standardized test meal is essential for the diagnostic accuracy of the 13C-UBT for the pre- and post-treatment *H. pylori* detection.

Based on a positive PCR analysis, 27% of the 13C-UBTs are incorrectly classified as false by other established reference tests. This finding lends support to the value of 13C-UBT in conditions of low bacterial density.

The addition of citric acid as suggested by the authors is critical for obtaining an optimal test result with the 13C-UBT. It reduces the necessary amount of the stable isotope 13C-urea, which is the principal cost factor. Citric acid has long become standard for the use of 13C-UBT.[15]

Unfortunately, patients with only one positive test in either RUT or histology were excluded from analysis in this study. This subset of patients is not infrequently encountered in clinical practice, and most likely several of these patients should then be classified in the category ''occult *H. pylori*''-positive individuals as well. In clinical practice a single positive *H. pylori* test should be considered sufficient for the initiation of eradication therapy. Coccoid forms of *H. pylori* frequently detected in the environment may develop as well in the human stomach and since they are metabolically dormant molecular tests need to be implemented in certain conditions.[16]

An apparently totally ''resolved'' field such as the diagnosis of *H. pylori* with a complexity of tests remains an interesting open forum for basic and translational research.

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Conflict of interest

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